Advances in Thermal and Non-Thermal Food Preservation
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This book is dedicated to food processors, engineers, researchers, entrepreneurs, and students who will take the ongoing research in food preservation to another level in the years to come.
Contents

Preface ix
Contributors xi

1 Basic Food Microbiology, 3
   Sadhana Ravishankar and Nicole Maks

Part One: Thermal Food Preservation
2 Thermal Processing of Liquid Foods with or without Particulates, 35
   Gaurav Tewari
3 Aseptic Processing, 43
   Rakesh K. Singh
4 UHT and Aseptic Processing of Milk and Milk Products, 63
   Nivedita Datta and Hilton C. Deeth
5 Microwave and Radio-Frequency Heating, 91
   Gaurav Tewari
6 Novel Thermal Processing Technologies, 99
   Antonio Vicente and Inês Alexandra Castro
7 Radio-Frequency Heating: Commercial Developments, 131
   Gaurav Tewari
8 Sous Vide and Cook-Chill Processing of Foods: Concept Development and Microbiological Safety, 145
   Vijay K. Juneja and Oscar P. Snyder

Part Two: Non-Thermal Food Preservation
9 Active Packaging: A Nonthermal Process, 167
   Jung H. Han and John D. Floros
10 The Ozonation Concept: Advantages of Ozone Treatment and Commercial Developments, 185
    John S. Novak and James T.C. Yuan
11 Electronic Pasteurization, 195
    Suresh D. Pillai and Leslie A. Braby
12 High-Pressure Processing of Foods, 203
    Gaurav Tewari
13 Pulsed Electric Field Technology: Effect on Milk and Fruit Juices, 241
    Hilton C. Deeth, Nivedita Datta, Alexander I.V. Ross, and Xuan T. Dam

Index 271
Preface

Food preservation has been a long-lasting desire of human beings. The significant developments in food preservation started the day fire was discovered by prehistoric humans, which was followed by indigenous methods of food preservation such as pickling, oiling, and salting of different food types, whether raw or processed. Some of the earlier techniques are still in use and are available in several commercial formats. The major developments and needs in food processing and preservation started during wars, when extended shelf life of foods became a necessity. As a matter of fact, several food processing techniques—such as ready-to-eat food in pouches, aseptic processing of milk and liquid foods with particles, and ohmic/electric resistance heating of foods—were developed to achieve extended shelf life of foods for soldiers in wars. A transfer of technology occurred when consumers started demanding a food product with fresh-like characteristics, along with extended shelf life. Over the years, consumers became more and more educated about adding food preservatives and their adverse effects on long-term health. This all added to research and development and finally the commercialization of innovative food preservation techniques. This also gave birth to several non-thermal food preservation techniques, including ultra-high-pressure processing, which has begun to see commercialization since the late 1990s. Irradiation is also getting limited acceptability by consumers for several food products. Thermal food preservation techniques are being revisited and are being modified to provide consumers with a variety of food products with home-cooked meal characteristics.

Researchers not only in the United States but also all over the world have played a major role in the latest developments of food preservation techniques. Europe and Asia have seen more commercial food products with extended shelf life on the shelves of their grocery stores than in the United States. Due to stringent regulatory control in the United States and reluctant behavior of established U.S. food processors in adapting new food preservation techniques, several food preservation techniques are still in their commercial infancy. The ones gaining limited commercial success are ultra-high-pressure processing, retort pouch technology, and, to some extent, irradiation.

Despite the availability of different food processing and preservation techniques discussed in a plethora of books available in stores, a single book with special attention to advancement in thermal and non-thermal food preservation and with special emphasis on commercialization of food preservation techniques was still needed. That is this book.
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Advances in Thermal and Non-Thermal Food Preservation
1 Basic Food Microbiology

Sadhana Ravishankar and Nicole Maks

Introduction

Microorganisms are present everywhere, and food is no exception. Microorganisms are commonly found in raw commodities and can get introduced into many processed commodities. Some microbes are beneficial in that they cause desirable changes in the food through the process of fermentation. Some cause undesirable changes in the foods that lead to spoilage and such products often become nonpalatable. Some other microorganisms cause human health risk in the form of foodborne diseases or food poisoning, when the food containing these microorganisms is ingested. The science of understanding these three different types of microorganisms is called food microbiology.

Food microbiology is an old science. Its history dates back to 1658, when a monk named Kircher, using a microscope, observed minute worms in spoiled meat and milk as well as in decaying bodies. Kircher described these as worms invisible to the naked eye. A few other notable mentions in the early food microbiology era also include Antonie van Leeuwenhoek, Lazaro Spallanzani, Nicolas Appert, and Louis Pasteur, among others. Leeuwenhoek was also one of the earliest persons to observe microorganisms under the microscope. Spallanzani was the first to show “sterility” of a thermally processed product. He showed that when meat broth was boiled for an hour and the container was sealed immediately after boiling, the broth did not spoil, and the appearance of microorganisms was prevented. Appert and Pasteur are the fathers of thermal processing. Appert developed the canning process. Pasteur is called the father of food microbiology since he based his discovery on science, and the process of pasteurization was named after his discovery of thermal processing of wine to inactivate undesirable microorganisms in the 1880s. Pasteur was also the first to discover food spoilage by showing that souring of milk was caused by microorganisms. He also showed that foodborne microorganisms were capable of causing diseases. Food microbiology has been developing since then as a science, and new foodborne disease causing agents have been emerging from time to time. In recent years, the foodborne disease reporting system has improved, and these illnesses are tracked very well by the Centers for Disease Control and Prevention (CDC) and other health agencies. The CDC, in collaboration with the U.S. Department of Agriculture (USDA), the U.S. Food and Drug Administration (FDA), and ten states (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee), developed a project called Foodnet (Foodborne Disease Active Surveillance Network). This project will help public health officials better understand the epidemiology of foodborne diseases, estimate the frequency and severity of these illnesses, and identify what foods are involved (CDC 2000a). The CDC has also developed another project, called PulseNet, for identification of foodborne disease causing bacteria through DNA fingerprinting. PulseNet is a national network of public health laboratories that are connected to a central electronic database at the CDC and can do a comparison of fingerprint patterns for identification of foodborne bacteria (CDC 2000b). Both Foodnet and PulseNet are useful in investigations...
of foodborne disease outbreaks. These show the technological developments of food microbiology as a science.

It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead et al. 1999). Known pathogens cause 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths, while unknown agents are involved in 62 million cases, 265,000 hospitalizations, and 3,200 deaths (Mead et al. 1999). The annual economic losses attributed to foodborne diseases associated with medical costs, productivity losses, and business losses due to legal problems may be as large as $5 billion to $6 billion (CAST 1994). Foodborne disease causing agents can be classified into the following: bacteria, viruses, protozoans, toxins, and prions. Food spoilage agents are predominantly bacteria, yeasts, and molds. There are several factors, both in the food product and in the environment, that can affect the growth or survival of foodborne spoilage and pathogenic agents in foods, and food microbiologists develop appropriate measures to control these microorganisms in foods. In this chapter the following topics will be discussed: factors influencing growth and survival of various spoilage and pathogenic agents in foods, and methods (processing of food) to control these microorganisms in foods.

Factors Affecting Growth of Spoilage and Pathogenic Microorganisms in Food

Microorganisms in foods can originate from different sources, including plant parts such as fruits and vegetables, animals, birds, seafood, air, soil, sewage, water, humans, food ingredients such as spices and other additives, various food contact surfaces such as equipment, and other miscellaneous sources such as packaging materials, containers, flies, and rodents (Ray 2001). Various factors affect growth and survival of microorganisms in foods. These factors could be intrinsic such as properties of the food or the microorganism itself, or extrinsic such as those of the environment.

Intrinsic Factors

Acidity, pH, and Buffering Capacity

Most bacteria do not tolerate high acidity. Use of fermentation for food preservation is based on this ability of acids to inhibit microbial growth. Weak organic acids such as acetic, lactic, citric, and malic acids have a better antimicrobial action compared to inorganic acids such as hydrochloric acids, and hence such organic acids are more commonly used in foods and are produced during food fermentations. Acids can also interact with other factors or hurdles in inhibiting microbes. The pH is a function of the hydrogen ion concentration in the food. Foods with a pH below 4.6 are called high-acid foods and those with a pH above 4.6 are called low-acid foods. This limit was set because in foods below pH 4.6, Clostridium botulinum spores cannot sporulate and produce toxin. The optimum pH for growth of microorganisms is close to neutral (pH 7), and most bacteria do not grow below pH 4.6. Bacteria are more fastidious in their relationship to pH than molds and yeasts, with pathogenic bacteria being the most fastidious (Jay et al. 2005). In general, the optimum pH range for bacteria is 6.0–8.0, for yeasts it is 4.5–6.0, and for filamentous fungi 3.5–4.0 (Adams and Moss 2000). The pH range for growth of molds is 1.5–9.0, for
yeasts 2.0–8.5, for Gram positive bacteria 4.0–8.5, and for Gram negative bacteria 4.5–9.0 (Ray 2001). The buffering capacity of a food refers to its ability to resist any changes in pH; hence, this ability should be considered when acidifying a food with various acids or fermenting a particular food for preservations. Fruits and vegetables usually have a low buffering capacity compared to muscle foods. The high protein content of muscle foods aids in their buffering abilities. Fruits usually undergo more spoilage from molds and yeasts than bacteria, since yeasts and molds can grow at pH below 3.5. Alkalinity with a pH of 11 or above is also detrimental to bacterial growth.

**Water Activity**

Dry foods are believed to be free from dangerous microorganisms and safe for human consumption, because microorganisms cannot grow and proliferate under dry conditions and they need free water for growth. The water activity of a food is defined as the ratio of the water vapor pressure of the food to the vapor pressure of pure water at the same temperature (Jay et al. 2005). Pure water has a water activity of 1.00. The optimum water activity for growth of most bacteria is above 0.92. The lowest water activity at which most food spoilage bacteria can grow is about 0.90 (Fontana 2000). *Staphylococcus aureus* can grow at water activities as low as 0.86. In general bacteria require higher water activity values for growth than fungi, and Gram negative bacteria have higher requirements for water activity than Gram positive bacteria (Jay et al. 2005). The lowest limit for growth of yeasts and molds is a water activity of 0.60 with no microbial proliferation occurring below 0.60 (Beuchat 1983). The minimum water activities required for active growth of most Gram negative bacteria, most Gram positive bacteria, most yeasts, most filamentous fungi, halophilic (salt tolerant) bacteria, and xerophilic (tolerant to dry environments) fungi are 0.97, 0.90, 0.88, 0.80, 0.75, and 0.61, respectively (Adams and Moss 2000). Water activity is usually used as a preservative factor by the addition of salt (for pickling of vegetables, meat, and fish) and sugar (for fruit preserves, jams, and jellies) (Forsythe 2000). The lowest water activity for any microbial growth is 0.60 and below this value the spoilage of foods cannot be microbiological but may be chemical or due to insect damage (Adams and Moss 2000). The water activity can also interact with other factors such as pH, acid, and nutrients and either inhibit or promote bacterial growth.

**Redox Potential**

Redox potential (Eh) or the oxidation-reduction potential is the measurement of the ability of a substrate to gain or lose electrons. Redox potential is defined as the ratio of the total oxidizing power (electron accepting) to the total reducing (electron donating) power of a food (FDA 2001b). When electrons are transferred between two compounds, a potential difference is created between them. This difference that can be measured by an instrument and expressed in millivolts (mV) is redox potential (Jay et al. 2005). Highly oxidized products will have positive Eh values and highly reduced products will have negative Eh values. Generally aerobic bacteria can tolerate higher Eh values better than anaerobes that require negative Eh values for growth. Microaerophiles such as lactobacilli and *Campylobacter* can thrive well under slightly reduced conditions. The various ranges of Eh for microbial growth are +500 to +300 mV for aerobes, +300 to −100 mV for facultative anaerobes, and +100 to −250 mV for anaerobes (Ray 2001). Microbial growth in general
in a food reduces the Eh values since oxygen is depleted and reducing compounds such as hydrogen are produced by microorganisms (Adams and Moss 2000). The growth of microorganisms at certain Eh values can also be influenced by other food components such as presence of salts, the poising capacity (resistance to changes in Eh) of food, processing treatment that a food undergoes, and presence of active respiratory enzyme systems (FDA 2001b). Fresh foods derived from plants or animals are usually reduced due to the presence of acids, reducing sugars, and −SH groups of proteins and upon diffusion of oxygen become more oxidized (Ray 2001). Aerobic bacteria utilize the O₂ in the medium as they grow and can lower the Eh values of the environment while anaerobes do not.

**Presence of Nutrients**

The nutrients present in a food product affect microbial growth because microorganisms need certain nutrients such as carbohydrates, proteins, fats, vitamins, and minerals for growth and metabolism. *Salmonella Enteritidis* has a need for iron to grow well (Clay and Board 1991). Microbes derive their energy mainly from carbohydrates, alcohols, amino acids, and fats. Amino acids and proteins are the major source of nitrogen for microorganisms. Other nonprotein nitrogen sources are urea, ammonia, creatinine, and trimethylamine (Ray 2001). Some of the minerals required for microbial growth include phosphorus, calcium, iron, sulfur, manganese, and potassium in small amounts, which are present in most foods (Ray 2001). Depending on the nutrient content of the food, those microorganisms that can metabolize the most available substrate will likely dominate in that food. Plant-based foods in general are rich in carbohydrates, some proteins, vitamins, and minerals, while animal-based foods are more abundant in proteins, lipids, vitamins, and minerals. Some microorganisms are more fastidious in their nutritional requirements than others. In general, Gram positive bacteria are more fastidious in their nutritional requirements (Jay et al. 2005). *Staphylococcus aureus* and *Listeria monocytogenes* require certain B vitamins for growth (Jay et al. 2005).

**Biological Structures of Food**

Many raw foods have an outer covering that is a natural barrier to microbial entry and growth. Some such structures are shells of nuts and eggs, fruit and vegetable skins, hides of animals, outer coverings or husks of grains, and the testa of seeds. These outer barriers are usually composed of macromolecules relatively resistant to degradation, provide an inhospitable environment for microbes with characteristics such as a low water activity and low readily available nutrients, and often contain antimicrobial compounds such as short chain fatty acids on animal skin and essential oils on plant surfaces (Adams and Moss 2000). As long as the outer covering is intact, internal contamination will be prevented. However, these outer barriers can become damaged during transportation, handling, processing, and storage, allowing microbial entry and subsequent growth under suitable conditions. For example, once there is a small crack on the egg shell, microorganisms can enter and grow under favorable conditions. Damaged fruits and vegetables spoil more quickly than nondamaged ones. The maturity of plant foods can influence the effectiveness of the protective barriers (FDA 2001b). For example, the skin of very ripe fruits is softer and more prone to damage than nonripe fruits that have a firmer skin and pulp.
Antimicrobials (Natural and Added)

Antimicrobials, whether present naturally or added as preservatives, can affect microbial growth in foods. Many foods have their own inherent antimicrobial properties through certain antimicrobial compounds present in them. For example, certain spices contain essential oils that have antimicrobial properties. Some plant antimicrobial compounds include eugenol, thymol, carvacrol, cinnamic aldehyde in certain spices, allyl isothiocyanate in cruciferous vegetables, allicin in garlic and onion, phaseollin in green beans, oleuropein in olives, flavonoids in some fruits and tea, and caffeic acid in chicory root. Animal-based foods also contain antimicrobials. For example, milk contains lysozyme, conglutinin, lactoferrin, and a lactoperoxidase system; and eggs contain lysozyme, avidin, ovotransferrin, ovoflavoprotein, and conalbumin. Ovotransferrin, avidin, and ovoflavoprotein are proteins that bind to nutrients essential for bacterial growth and thereby prevent growth of microorganisms requiring these nutrients. Often the processing of foods can introduce antimicrobial compounds. Fermentation is one such process, wherein compounds such as hydrogen peroxide, bacteriocins, carbon dioxide, reuterin, diacetyl, or others are produced as metabolites of lactic acid bacteria that can inhibit other microorganisms. Smoking can introduce compounds such as phenols that are inhibitory to microbes. Maillard reaction compounds have antimicrobial properties (Mossel et al. 1995). A number of chemical preservatives are used as additives in foods and are approved for food use by FDA. Salts of organic acids such as lactates and diacetates are used in ready-to-eat meats and have inhibitory activity against certain foodborne pathogens. A combination of pediocin, sodium lactate, and sodium diacetate was found to have inhibitory activity against L. monocytogenes in frankfurters (Uhart et al. 2004). Sorbates and benzoates are used as preservatives against yeasts and molds.

Extrinsic Factors
Storage Conditions

The conditions under which a food is stored can greatly play a role in microbial growth. Among those conditions, temperature, time, and relative humidity of storage environment are important and can significantly affect microbial growth and survival. These extrinsic factors can also interact among themselves as well as with other intrinsic factors such as pH and water activity and can influence growth of microorganisms. Understanding such interactions is important in selecting the proper storage conditions for various food products.

Temperature of Storage

Microorganisms can grow over a broad range of temperatures. However, the optimum temperature of growth for most foodborne pathogens is between 30 and 37°C. Microorganisms can also grow at low temperatures but slowly. Based on their growth temperature ranges, microorganisms can be classified as psychrophiles (cold loving; optimum between 12 and 15°C), mesophiles (moderate temperature loving; optimum between 30 and 40°C), and thermophiles (heat loving; optimum between 55 and 65°C). Some of the mesophiles can also grow at low temperatures and are called psychrotrophs (growth at 0 to 5°C; optimum between 10 and 30°C). Many food spoilage microorganisms are psychrotrophs. Examples of psychrotrophs are molds, yeasts, Pseudomonas, Yersinia, Serratia, Aeromonas, Listeria,
*Clostridium*, *Bacillus*, *Leuconostoc*, and *Lactobacillus*. Most foodborne pathogens are mesophiles. Few foodborne microorganisms are thermophiles. One example is *Bacillus stearothermophilus*, a Gram positive sporeformer, which can cause spoilage of canned foods. The temperature of storage can greatly affect the lag phase as well as the exponential growth phase of a microorganism.

**Time of Storage**

The time of storage of a food product is determined by the product shelf life. That is why most retail products have an expiration date or a sell by date beyond which the product may not be safe to consume or in some cases not palatable. The time of storage also depends upon other extrinsic and intrinsic factors that affect microbial growth. When time alone at ambient temperature is considered as a factor for controlling microbial growth, it is better to hold the product for less than or equal to the lag phase of the foodborne pathogen of concern in that product (FDA 2001b). The time and temperature of holding is also important in cooling of cooked products since the growth of pathogens such as *Clostridium perfringens* is known to occur during improper or slow cooling of foods prepared in large quantities and stored in huge containers. Even if foods are prepared/cooked in large quantities, they need to be cooled promptly by storing in smaller containers for ease and speed of cooling. For most food products, time and temperature of storage go together and both should be considered essential for microbiological safety of a food product.

**Relative Humidity of Storage**

The relative humidity of a storage environment may alter the moisture content of a food product. The higher the relative humidity of the environment, the greater the chances for the water activity of the product to increase due to the exchange of moisture from the environment into the food product, and this may have a negative effect on the product shelf life. If the change in water activity is such that microbial growth is permitted, it could cause spoilage or render the food unsafe due to growth of pathogens. For example, improperly wrapped meats tend to undergo surface spoilage more quickly due to the high relative humidity in the refrigerator (Jay et al. 2005). Hence, storage at appropriate relative humidity is important. If the storage environment is dry or less humid and the product loses moisture, it may not affect the microbiological safety of the product; however, this may have negative effects on the sensory attributes. It is better to store foods that can easily undergo surface spoilage from molds, yeasts, or bacteria under conditions of lower relative humidities. Using gases could be an alternate measure to prevent surface spoilage in packaged foods.

**Type of Packaging and Packaging Atmosphere**

The type of packaging and packaging atmosphere can greatly influence microbial growth. Often gases are used in packages since they can inhibit microorganisms. For example, CO₂ could be lethal to aerobic microorganisms, and O₂ can inhibit anaerobic growth. Ozone is another gas used for sanitizing the surface of fresh produce as well as equipment. Ozone at 0.15–5.0 ppm concentrations has been shown to inhibit spoilage bacteria as well as yeasts (Jay et al. 2005). Other gases in vapor form are also used for sanitation purposes.
Various types of packaging using gases for preservation include modified atmosphere packaging (MAP), controlled atmosphere storage (CA), direct addition of CO₂ (DAC), and hypobaric storage (Loss and Hotchkiss 2002). The gases usually used in MAP include CO₂, O₂, and N₂ in various proportions depending on the type of product and microorganism of concern. The solubility of these gases depends on various other factors such as the storage temperature and food composition. For example, CO₂ has increased solubility at low temperatures and low salt concentrations (FDA 2001b). With modified atmosphere the interactions of various factors such as temperature, product to headspace gas volume ratio, package barrier properties, type and load of the microflora, and food composition play a major role in microbial inhibition (Loss and Hotchkiss 2002).

Processing and Packaging

The type of processing a food undergoes is one factor that determines the microbiological safety of the product. For example, canned foods are processed to a temperature high enough to inactivate vegetative cells as well as spores of pathogens and most spoilage bacteria and thus can be stored at room temperature for a long period of time. On the other hand, minimally processed foods have a shorter shelf life. Packaging after processing is important since recontamination of processed foods needs to be prevented. A good package should be able to prevent microbial entry. Handling of the products after processing and prior to packaging should be done carefully to avoid recontamination of the product. During processing, time and temperature of processing are important. If the required temperature and time are not reached, the process will be inadequate, allowing growth of surviving microorganisms.

Other Factors

Actions and Implicit Factors of Microorganisms

Raw foods are not sterile and can harbor a variety of microorganisms. The types that dominate can depend on the numbers present, the utilizable substrates present in the food, and the accumulation of metabolite products. Some bacteria can grow in the presence of other bacteria, having a synergistic interaction, while some others cannot, having an antagonistic interaction. Certain compounds such as bacteriocins produced by certain bacteria or acids produced by lactic acid bacteria can inhibit coliforms and promote growth of succeeding flora required for fermentations. In sauerkraut fermentations, leuconostocs grow initially and produce lactic acid, which lowers the pH of the product, and this allows growth of lactic acid bacteria that can bring about changes associated with product flavor. At times some microflora are able to utilize nutrients required for growth of some others and compete better. For example, coliforms and *Pseudomonas* utilize amino acids and streptococci utilize certain vitamins required for *Staphylococcus aureus*, thereby inhibiting the growth of this pathogen (ICMSF 1980). In some instances, the growth of one microorganism can remove the inhibitory component and allow growth of another microorganism. For example, in mold ripened cheeses, mold growth can increase the pH, allowing pathogens such as *L. monocytogenes* to grow, which could compromise the safety of these products. The physiological state of a bacterium can affect its growth and survival. For example, exponential phase cells are easier to inactivate by many processes or treatments than stationary phase cells. Also, cells preadapted to certain stresses can resist some lethal stresses better than
nonadapted cells. Acid-adapted cells of *L. monocytogenes* survived better at low pH values of 4.0 and 3.5 than non-acid-adapted cells (Ravishankar and Harrison 1999), and acid-adapted cells were more tolerant of an activated lactoperoxidase system than nonadapted cells (Ravishankar et al. 2000). Cell to cell signaling and secretion of proteins have been known to occur in microbial populations as a warning of stress to the neighboring cells.

**Preparation and Handling by the Consumer**

The way a consumer prepares or handles a food product can affect its microbiological stability. Some products may require a particular temperature of storage once the packaging is opened, and consumers should be made aware of this. Many shelf-stable products held at room temperature need refrigeration once opened. Also, proper handling of the foods by consumers is important to avoid cross contamination, especially for foods that are consumed without further treatment. For example, the cutting boards and knives used to chop or slice raw meat should not be subsequently used to chop salad vegetables without further cleaning, since this could cause contamination of salad vegetables with harmful microorganisms that may be present in raw meat. It is always better to consume the food products by or before their expiration date or sell by date.

**Product History and Traditional Use**

The previous history and traditional use of a product tell about the microbiological safety of a product. For example, a product that has been implicated in a foodborne disease outbreak needs to be handled or processed and stored carefully to avoid such occurrence again. However, if there is any change in the product end use, processes, formulation, physical structure, processing, distribution, or storage, the storage time and temperature need to be revalidated, and history cannot be used as the basis for determining safety (FDA 2001b). Also, it should be considered if the food product or one of its ingredients has been involved historically as a vehicle of foodborne disease outbreaks due to abusive handling or storage at ambient temperature, or if adequate temperature control of the product has been the sole factor for preventing foodborne illness (FDA 2001b).

**Microbiology of Food Spoilage**

Spoilage refers to changes in a food product that make it sensorially unacceptable for human consumption. The change could be either physical damage (change in viscosity), chemical reactions (oxidation, pH or other changes), microbiological (off-odors, off-flavors, off-colors, slime formation), or changes due to insect and rodent damage. Microbial spoilage is more common than chemical spoilage, and it is estimated that 25% of the global food produced annually is lost postharvest or postslaughter due to microbial spoilage (Anonymous 1985). Food spoilage is a worldwide problem. In the developed nations, spoilage due to psychrotrophs, yeasts, and molds is predominant, while in developing nations insect and rodent problems are more common (Huis in’t Veld 1996). The reason could be that in the developed nations refrigeration facilities are commonly available, and that is not the case in developing nations where prepared food is consumed immediately and not stored for longer periods. Spoiled food is not poisonous and, therefore, spoilage is a quality issue, not a safety issue (Forsythe 2000). However, the presence of
large numbers of certain indicator microorganisms that can cause spoilage may also be indicative of the presence of certain pathogens.

Food spoilage has been studied extensively and microorganisms causing food spoilage in different types of foods have been well characterized by food microbiologists. Though both foodborne pathogens and spoilage microorganisms undergo similar kinds of stresses in foods, spoilage microorganisms tend to withstand harsh conditions better, often develop resistance to chemical preservatives and sanitizing agents, and are able to outnumber foodborne pathogens both in quantity and types (Roller 1999). Gram et al. (2002) describe three different types of interactions as survival strategies among food spoilage bacteria. One type of interaction is antagonism caused as a result of competition for nutrients (such as iron that can be mediated by bacterial siderophore production), and there is subsequent suppression of less competitive species. The second type of interaction is called metabolism, whereby there is a change in the spoilage profile of a microorganism due to the supply of nutrients from another microorganism present in the same food. The third type of interaction is bacteria cell to cell communication in which certain Gram negative bacteria are able to coordinate certain phenotypic traits such as hydrolytic enzymes by communication via N-acyl homoserine lactones.

During food spoilage, microbial growth produces certain metabolites causing biochemical changes in the food product. Dainty (1996) has described a number of compounds including volatile fatty acids, indole, hydrogen sulfide, metabolites of sorbates, 4-vinylguaiacol, D-alanine, gluconic and 2-oxoglucunic acids, L- and D-lactic acids, acetic acid, ethanol, biologically active amines, methane, trimethylarsine, and other volatile compounds as metabolites produced during spoilage of food by microorganisms. Microorganisms produce enzymes in foods and the action of these enzymes can cause changes in the spoiled food. Pseudomonas can produce proteases and lipases, which can cause off-flavors in milk and other dairy products (Frank 1997). Whitfield (1998) has given an excellent review of the compounds responsible for off-odors and off-flavors produced in foods during microbiological spoilage. Along with the compounds, the specific microorganism responsible for formation of the compounds has also been described for most spoilage examples. This review clearly implies that for detection of specific spoilage, it is important to understand what kind of metabolites are associated with which microflora (Whitfield 1998). Also, the relationship between chemical and microbial spoilage needs to be better understood.

Methods for detection of specific spoilage microorganisms include spoilage potential, spoilage activity, yield factor determination, and chemical spoilage profiles (Dalgaard 1993). Each of these methods is based on correlating the bacterial concentrations with the off-odors or metabolites formed in the spoiled products. In chemical spoilage profiles, comparison of natural spoilage is done with controlled or experimental microbial spoilage. Foods that are high in nutrient content, especially proteins, have a pH close to neutral (low acid food) and a high moisture content, and hence, tend to spoil faster than nonproteinaceous foods or foods with low protein content. Also, some foods that do not undergo processing treatment such as fresh foods (seafood and meats) spoil faster than processed foods since they may have an initial load of spoilage microflora (in low numbers), which can grow and increase during storage, causing off-flavors, off-odors, or slime and spoilage. According to Forsythe (2000), on the basis of spoilage susceptibility, foods can be categorized as nonperishable or stable (for example, flour due to low water activity), semiperishable (for example, apples can spoil slowly due to improper handling and storage), and per-
ishable (for example, raw meat, due to high water activity and high pH). The growth of some spoilage microorganisms can be prevented by refrigeration and certain special types of packaging such as modified atmosphere packaging and vacuum packaging. Refrigeration, however, cannot completely prevent spoilage since the psychrotrophic microorganisms can still grow but rather slowly. The psychrotrophic microflora are comprised of bacteria, yeasts, and molds; however, yeasts and molds in general do not compete very well with bacteria except in situations where bacteria cannot predominate; for example, in foods with high sugar or acidity (Huis in’t Veld 1996). The food spoilage microorganisms can be broadly classified as Gram negative bacteria, Gram positive (sporeformers, lactic acid bacteria, and others) bacteria, yeasts, and molds.

**Gram Negative Food Spoilage Bacteria**

Generally foods with pH close to neutral, high in moisture content, and stored aerobically undergo spoilage due to Gram negative bacteria. Among the Gram negative bacteria, *Pseudomonas* is the most predominant spoilage bacterium. It can cause psychrotrophic spoilage in a variety of foods such as milk, red meat, poultry, and seafood. *Pseudomonas* is widely present in the environment and can contaminate foods from several sources and utilize a variety of substrates (Huis in’t Veld 1996). Spoilage by *Pseudomonas* can result in slime formation and pigments on the spoiled food (Dainty 1996; Dainty and Mackey 1992) and off-flavors and off-odors, especially in animal products due to metabolism of nonproteinaceous nitrogenous compounds (Huis in’t Veld 1996). Other psychrotrophic Gram negative bacteria include *Aeromonas*, *Shewanella*, *Vibrio*, *Hafnia*, *Moraxella*, *Serratia*, *Flavobacterium*, *Erwinia*, and *Pantoea*. Vibrios are common spoilage microorganisms in fish and other seafood. *Erwinia carotovora* and *Pseudomonas* are responsible for about 35% of spoilage of vegetables (Forsythe 2000). *Pseudomonas* can cause postprocess contamination of pasteurized milk (Eneroth et al. 2000). Off-odors in pasteurized milk are produced by *P. putida* and *Yersinia intermedia* (Whitfield et al. 2000). At temperatures above 10°C, nonpsychrotrophic bacteria such as those belonging to the *Enterobacteriaceae* family will predominate and cause spoilage. In these cases, there is production of acid, gas, slime, rope, bitter flavors, and off-odors (Huis in’t Veld 1996). Due to its red pigmentation, *Serratia marcescens* can cause “bloody bread” in bread (Brackett 1997).

**Gram Positive Food Spoilage Bacteria**

The Gram positive psychrotrophic spoilage microflora include species of *Brochothrix*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, and *Vagococcus*. *Carnobacterium piscicola* is associated with fish spoilage. *Brochothrix thermosphacta* is found in raw meats, and MAP, as well as vacuum packaging, allows the growth of this microorganism (Huis in’t Veld 1996). Enterococci are often used as indicator microorganisms. Lactobacilli and lactococci cause fermentations in the foods, which may be desirable for some foods. However, fermentations producing acids and other metabolites may be undesirable in some foods such as vacuum-packaged meat, poultry, and cured meats (Borch et al. 1996; Dainty et al. 1983). Lactic acid bacterial fermentation is also undesirable in fruit juices. For example, *Lactobacillus kunkeei* causes spoilage of grape juice through fermentation (Edwards et al. 1998). *Micrococcus* is a relatively heat-resistant microorganism, is present in raw milk in low numbers, and can survive pasteurization and
cause spoilage of milk. Beer spoilage called “rope” is caused by *Pediococcus* and *Acetobacter*, and lactic acid bacteria produce diacetyl in beer, which gives an undesirable flavor (Forsythe 2000). Lactobacilli are specific spoilage microorganisms identified in beer (Sakamoto et al. 2001), and *Pediococcus* species are important beer-spoiling microorganisms (Jespersen and Jakobsen 1996). *Leuconostoc, Lactobacillus, Streptococcus, Micrococcus*, and *Bacillus* were isolated from spoiled doughs, with the majority of the microflora being *Leuconostoc* (Elliott 1980).

**Spoilage by Sporeformers**

The major sporeforming spoilage microorganisms belong to the genera *Bacillus* and *Clostridium*. Some strains of these microorganisms can cause psychrotrophic food spoilage. For example, psychrotrophic *Clostridium* causes spoilage of vacuum-packed beef and ham (Dainty 1996) and vacuum-packed cooked beef and pork (Hansen et al. 1995; Lawson et al. 1994). Psychrotrophic *Bacillus weihenstephanensis* can cause “sweet curdling” of refrigerated pasteurized milk (Jay et al. 2005). Psychrotrophic *Bacillus cereus* can cause sweet curdling and “bitty cream” in pasteurized milk stored under refrigeration conditions (Forsythe 2000). Sweet curdling is coagulation without significant acid or off-flavor and is caused by protease produced by the spoilage bacterium (Frank 1997). Bitty cream is formed due to the degradation of fat globule membrane by lecithinase (produced by *Bacillus cereus*), resulting in aggregation of fat in the cream (Frank 1997). Psychrotrophic *Clostridium* causes “blown pack” spoilage of vacuum-packed meats (Broda et al. 1996). *Clostridium tyrobutyricum* and occasionally *C. sporogenes* and *C. butyricum* cause gas formation called “late blowing” or “late gas” in aged cheeses (Frank 1997). *B. subtilis* and *B. licheniformis* can cause “ropiness” in bread, which is characterized by a stringy brown mass within the bread loaf (Brackett 1997). *Alicyclobacillus acidoterrestris* is an acid-tolerant sporeforming bacterium causing spoilage in fruit juices (Walls and Chuyate 2000). *Bacillus* and *Clostridium* also cause spoilage of canned food products. Some of the sporeforming bacteria causing spoilage of canned foods are *Bacillus stearothermophilus, Bacillus coagulans, Bacillus polymyxa, Clostridium thermosaccharolyticum*, and *Desulfotomaculum nigrificans* (formerly called *Clostridium nigrificans*). These mainly cause spoilage of canned foods arising out of underprocessing or damage to the can. *B. stearothermophilus* and *B. coagulans* cause “flat-sour” spoilage. There is no bulging of the cans and acids are produced from carbohydrates present in the food product. *C. thermosaccharolyticum* and *B. polymyxa* cause “swells” in the can. The swell could be a “hard swell” or a “soft swell.” In hard swells the can bulges due to gas and explodes. There are two types of soft swells: “flipper” swells and “springer” swells, where one end of the can is bulged out. In flipper swells, if you press the bulged end, the other end pops out. In springer swells, if you press the bulged end, the other end does not pop out. *D. nigrificans* causes spoilage known as “sulfide stinker,” in which the spoiled product has the smell of rotten eggs. In canned condensed milk “sweet coagulation” similar to sweet curdling is caused by *B. coagulans, B. stearothermophilus*, and *B. cereus* (Frank 1997).

**Food Spoilage by Yeasts and Molds**

Yeasts and molds can spoil a wide variety of food products including those with low pH, high sugar or salt content, or low water activity. These microorganisms can act on a
number of substrates including carbohydrates, proteins, lipids, and organic acids (Huis in’t Veld 1996). Yeasts and molds cause spoilage of fruits, vegetables, and bakery products, and they produce pectinolytic enzymes that soften the plant tissues, causing rot (Forsythe 2000). The spoilage by yeasts and molds is most visible either in the form of spores or slime, often pigmented usually on the surface. Yeasts that can grow in high concentrations of sugar such as found in jams, jellies, and preserves are called osmophilic yeasts, and they can cause spoilage of these products. Saccharomyces and Torulopsis are examples of osmophilic yeasts. Yeasts associated with spoilage of dried fruits include Zygosaccharomyces rouxii, Hanseniaspora, Candida, Debaryomyces, and Pichia species (Brackett 1997). Zygosaccharomyces bailii can cause spoilage of salad dressings. Yeasts can spoil sweetened condensed milk and butter, and some yeasts involved in dairy product spoilage include Kluyveromyces marxianus, Debaryomyces hansenii, Candida famata, Candida kefyr, Rhodotorula mucilaginosa, Yarrowia lipolytica, and Candida parapsilosis (Fleet 1990; Frank 1997; Rohm et al. 1992). Molds causing spoilage of cheeses include Penicillium, Aspergillus, Alternaria, Mucor, Fusarium, Cladosporium, Geotrichum, and Hormodendrum (Frank 1997). Rhizopus, Penicillium, and Aspergillus can cause spoilage of bread. Mucor, Rhizopus, and Thamnidium cause spoilage of meat (Forsythe 2000). Thermally processed fruit products can be spoiled by molds such as Byssochlamys, Neosartorya, and Talaromyces (Brackett 1997). Molds can cause spoilage of grains and grain products as well as adverse changes in appearance (due to colored spores), flavor, and aroma (due to some volatile compounds) of these products and can cause an increase in the free fatty acid value (Brackett 1997).

Foodborne Disease Agents

Salmonella

Salmonella is a motile Gram negative rod-shaped bacterium. It is widespread in animals and environmental sources including water, soil, insects, and animal feces. The genus Salmonella is divided into two species, S. enterica and S. bongori with over two thousand serotypes, and S. enterica is subdivided in six subspecies where serotypes isolated from humans, agriculture, and foods usually belong to subspecies enterica (D’Aoust 2001). The two most widespread serotypes that cause human salmonellosis are Typhimurium and Enteritidis (CDC 2003).

The consumption of undercooked meats, poultry, dairy products, raw eggs, and egg products contaminated with Salmonella is often implicated in cases of salmonellosis. Other foods that have caused outbreaks include alfalfa sprouts, infant formula, orange juice, and ice cream (D’Aoust 2001). After the pathogen is ingested, it penetrates and passes from the lumen into the epithelium of the small intestine, which then becomes inflamed. It is estimated that 1.4 million cases of salmonellosis occur each year in the United States, with only approximately forty thousand being reported each year (CDC 2005). All age groups are affected, with young children, the elderly, and immunocompromised individuals most susceptible to severe infections. Symptoms of salmonellosis include fever, abdominal cramps, and diarrhea 6–48 hours after infection, with acute symptoms lasting 1–2 days. The infective dose can be as few as 15–20 cells and is dependent on age and health of the host (FDA 1998).
Listeria monocytogenes

*Listeria monocytogenes* is a motile Gram positive rod-shaped bacterium. It is a facultative anaerobe and can grow over a wide temperature range including refrigeration temperatures. *L. monocytogenes* can grow in the pH range of 4.39–9.4 and is salt tolerant. *Listeria* is ubiquitous in nature, occurring in soil, decaying vegetation, and water. Between 1 and 10% of humans may be intestinal carriers of *L. monocytogenes*, and it has been found in other mammalian and bird species (FDA 1998).

Although *L. monocytogenes* has been recognized as a human pathogen, it was not until the 1980s that it was known as a foodborne pathogen (Ryser and Marth 1999). *L. monocytogenes* is an intracellular pathogen that causes listeriosis. The bacterium invades the gastrointestinal epithelium; enters the host’s monocytes, macrophages, or polymorphonuclear leukocytes; becomes bloodborne; and grows. Because it is present in phagocytic cells, this permits access to the brain and transplacental migration to the fetus in pregnant women (FDA 1998). Most clinical isolates of *L. monocytogenes* belong to serotypes 1/2a, 1/2b, and 4b. Listeriosis occurs as sporadic disease and epidemic outbreaks. The CDC estimates that up to 2,500 cases and 500 deaths occur from listeriosis each year in the United States (Mead et al. 1999).

Some foods involved in listeriosis outbreaks include coleslaw, soft cheeses, raw milk, and ready-to-eat deli meats and hot dogs. Foods become contaminated with *L. monocytogenes* by using unpasteurized milk in products and by postprocess contamination. There is a “zero tolerance” policy for *L. monocytogenes*, meaning that ready-to-eat foods contaminated with *L. monocytogenes* at a detectable level are considered adulterated.

Immunocompromised individuals, the elderly, pregnant women, and neonates are most susceptible to listeriosis. The infective dose of *L. monocytogenes* is not known and may depend on the strain and an individual’s susceptibility, but it is thought that ingestion of fewer than one thousand microorganisms may cause disease (FDA 1998). In adults, listeriosis is characterized as invasive and noninvasive. Invasive illness, which has an onset time of a few days to 3 weeks, is characterized by septicemia, meningitis, and encephalitis. In pregnant women, intrauterine or cervical infections can occur, which may result in spontaneous abortion or stillbirth (FDA 1998). Noninvasive listeriosis has an onset time of 18–20 hours, is characterized by gastrointestinal symptoms such as nausea, cramps, vomiting, and diarrhea, and is accompanied by fever, malaise, and headache (Donnelly 2001).

Escherichia coli O157:H7

*Escherichia coli* is a Gram negative, facultatively anaerobic, rod-shaped bacterium. It is found in the lower intestine of warm-blooded animals. *E. coli* O157:H7 is a serotype referred to as enterohemorrhagic *E. coli* O157:H7 (EHEC). It was first recognized as a pathogen in 1982 (Riley et al. 1983). After a large multistate outbreak in 1993, it became recognized as an important threatening pathogen (Rangel et al. 2005). EHEC are acid tolerant and able to survive acidic conditions in foods and passage through the stomach.

There are approximately 25,000–73,000 cases of illness and 60–100 deaths due to *E. coli* O157:H7 each year in the United States (FDA 2001a; Mead et al. 1999). Some foods implicated in outbreaks are alfalfa sprouts, undercooked ground beef, lettuce, and unpasteurized juices. The infective dose may be as little as ten cells. *E. coli* O157:H7 produces
a verotoxin and causes severe damage to the lining of the intestine, hemorrhagic colitis that is characterized by acute abdominal cramps, and bloody diarrhea (FDA 1998). Vomiting is common but fever is rare. Illness onset is usually 3–4 days but ranges from 1–9 days, and illness lasts 2–9 days (Feng 2001). In about 2–15% of *E. coli* O157:H7 cases, hemolytic uremic syndrome (HUS) occurs (Dundas et al. 2001). HUS is characterized by renal failure and is more prevalent in children. Most children recover without permanent damage. In adults, HUS is accompanied by fever and neurological symptoms and is often referred to as thrombotic thrombocytopenic purpura and can have a mortality rate as high as 50% in the elderly (FDA 1998).

**Campylobacter jejuni**

*Campylobacter jejuni* is a Gram negative, slender, curved rod that is motile and microaerophilic. It is sensitive to drying, freezing, acidic conditions, and salinity, and therefore sensitive to environmental stresses. *C. jejuni* has been isolated from healthy cattle, chickens, birds, and flies and is sometimes present in streams and ponds (FDA 2001a). The intestines of poultry are easily colonized with *C. jejuni*. In commercial operations, most chickens are colonized within 4 weeks (Humphrey et al. 1993).

*C. jejuni* was first identified as a human pathogen in 1973, and it is the most diagnosed cause of human gastroenteritis with undercooked poultry and cross contamination from raw poultry being the major risk factors (Altekruse et al. 1999). The illness caused by *C. jejuni* is referred to as campylobacteriosis. There are an estimated 2.1 to 2.4 million cases of campylobacteriosis each year in the United States (Tauxe 1992). Most cases of campylobacteriosis are sporadic. Outbreaks have different epidemiological characteristics from sporadic infections and usually occur during the spring and autumn (Tauxe 1992). Raw milk, untreated water, and raw clams are some foods implicated in *C. jejuni* outbreaks (FDA 1998).

All individuals are susceptible to *C. jejuni*, but children and young adults are more frequently infected (FDA 1998). Those who become ill suffer diarrhea, abdominal pain, and cramping. Diarrhea may be bloody and can be accompanied by nausea and vomiting. Symptoms usually occur 2–5 days after exposure and last up to 1 week. There can be serious sequelae in some people. Guillain-Barré syndrome (GBS), a serious sequela of *C. jejuni*, is a demyelinating disorder that results in acute neuromuscular paralysis (Allos 1997). It is estimated that one case of GBS occurs for every one thousand cases of campylobacteriosis, and up to 40% of people with GBS have evidence of a recent campylobacter infection (Allos 1997). Also associated with campylobacteriosis is Reiter syndrome, a reactive arthropathy, in which joint pain can last for months or become chronic (Peterson 1994). Both GBS and Reiter syndrome are thought to be autoimmune responses caused by *C. jejuni* infection (Altekruse et al. 1999).

**Staphylococcus aureus**

*Staphylococcus aureus* is a Gram positive facultative coccus that grows best under aerobic conditions. Staphylococci can tolerate up to 10–20% salt and 50–60% sucrose, and growth may occur at a water activity as low as 0.86 under aerobic conditions and at 0.90 under anaerobic conditions. The optimum growth temperature is 35°C, but it can grow from 7 to 47°C. *S. aureus* can grow in a pH range of 4.5–9.3 with an optimum between pH 7.0 and